

Control of Leaf Stomatal Opening

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week #7 (18 October)

week #8 (25 October)

week #9 (01 November)

The opening and closing of stomata is a very important mechanism that plants use to control the diffusion of gases in and out of leaves. Ideally stomata must be sufficiently open to allow enough CO₂ (needed for photosynthesis) to diffuse in, but sufficiently closed to prevent too much evaporative loss of H₂O. This is sometimes a difficult balance to achieve and the amount of stomatal opening is controlled by a large number of factors.

The degree to which stomata are open can be observed and measured by making imprints of the leaf epidermis and viewing (and photographing) these imprints using a light microscope. Once this technique is mastered it can then be used to measure the effects on stomatal aperture of a wide variety of environmental and chemical factors. Epidermal imprints can be made by applying a dental resin to the surface of the leaf and allowing it to harden. This negative impression can be archived and used to make a positive image (with clear nail polish) whenever desired.

PART A – TECHNIQUES FOR ANALYSIS OF STOMATAL APERTURE

I. Procedure for making imprints of the leaf epidermis

1. When you have the plant material all ready to go, mix equal amounts of the dark green and light green pastes together to make up the working dental resin. Cover 2 to 3 cm of the end of a popsicle stick with a layer (about 3 mm thick) of the resin. Gently push the resin onto the surface of the leaf and hold it there for 3 to 4 minutes until the resin hardens. Gently pull the resin away from the leaf surface, leaving it attached to the popsicle stick. The part of the stick that is free of resin can be used to label the impression. Give the resin about 10 minutes to fully set before proceeding to the next step.

2. Brush a layer of clear nail polish onto the surface of the dental resin impression that you want to view with the microscope. Wait for the nail polish to dry completely (allow about 10 minutes).

3. Carefully peel off the nail polish impression and place it onto a microscope slide. If you want to preserve the slide for later use, you can place a cover slip over the top, and seal around the edges of the cover slip with clear nail polish. Be sure to properly label the slide so you will know what you are looking at.

Following the procedure outlined above, make epidermal imprints of both the upper (adaxial) and lower (abaxial) surfaces of a leaf from the greenhouse. Observe these imprints using a microscope. *Based on your observations, which of these surfaces will work better for your future studies of leaf stomata?*

Make epidermal imprints of a well-hydrated leaf, a leaf that is slightly wilted, and a leaf that is very wilted. Observe these imprints using a video microscope and *save a movie file of a representative area of each.*

II. Procedure for making digital records (movie clips) of epidermal surfaces.

1. Place your microscope slide onto the stage of one of the video microscopes that is connected to a computer and turn on the microscope's light source. On the computer, open up the "iMovie" program and close down all other programs. Create a folder with the name of your group and put it on the desktop of the computer.

2. At the bottom of the "movie screen" and to the left, make sure that you are in the "camera" rather than the "scissors" mode. The movie screen should now be blue and should say "Camera Connected". Choose **File → New Project**. Give your "movie" an name (*eg. marymovie1*) and save it. Click on the Import button below the movie screen, then click the stop (■ ■) button. You should now be able to observe the stomata in your epidermal impression on the movie screen. Move the slide around until you see some stomata showing on the screen. Use the focus knob on the microscope to obtain the sharpest possible focus of the guard cells on the computer screen. Please note that focusing while looking into the microscope may not give optimal focus on the computer screen. Observing the stomata at 100x magnification (using the 10x objective lens) is good for getting a general look at many stomata, while 400x magnification (using the 40x objective lens) is best for making actual measurements of individual stomata.

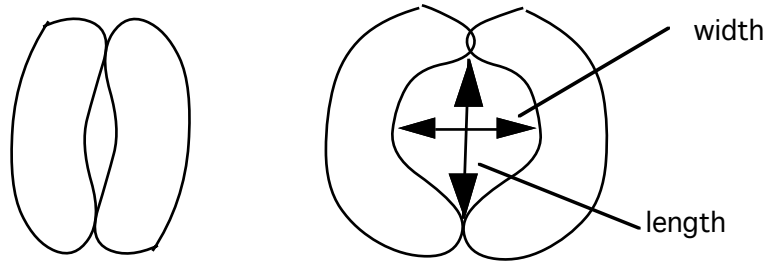
3. To record the images of stomata from a particular microscope slide, do the following: Set the microscope up at 400x magnification with a stoma in clear focus. Click the stop (■ ■) button, then the Import button to restart your "movie". As the computer is recording the movie, move your slide around on the microscope, bringing more stomata, one or two at a time, into the field of view. Make sure to clearly focus on each stoma, before moving the field of view on to the next one. When you have included a sufficient number (in this case about 20) of stomata, click the stop (■ ■) button to end the "movie clip".

Repeat this process until you have made "movie clips" of each of the leaves that you need to study. *Carefully record in your notebook which movie clips correspond to particular leaves/stomatal impressions.* Close and save your movie file. Open up the Fileserver1 (smb://fileserver1/) and copy your "movie" (which will include several "clips") into the BI214-2004 folder of the server.

Now you are finished with the video microscope. If another group needs to use the computer attached to the video microscope, you should move to a different computer to continue with your work.

III. Procedure for measuring stomatal aperture from digital images.

You are now ready to measure the apertures of the stomata in the 4 impressions that you made above. A commonly accepted way to measure stomatal aperture is to report the width of the opening divided by the length. Wide open stomata will have width/length values of 0.5 or more, while fully closed stomata will have a value of 0.



It is important to be able to recognize and correctly measure the stomatal opening. Of particular importance is the ability to distinguish between the flaps connected to the guard cells and the actual opening. Notice that in the **closed** stoma shown below, there is a distinct line visible between the two guard cells, indicating that the guard cells are in complete contact (thus there is no opening). In the two **open** stomata, it can be seen that the flaps of the two guard cells are not touching, leaving an opening for gases to travel through.



closed

partially
open

open

$$w/l = 0$$

$$w/l = 0.18$$

$$w/l = 0.29$$

To measure the apertures of the stomata in your impressions, you can use any one of the computers in Arey 305.

1. Copy the movie that you need from the Fileserver1 into the Plant Physiology folder on the hard drive of the computer you are using (NOT onto the desktop). Do NOT attempt to open any files directly from the Server.

2. Open iMovie and close all other applications. Make sure that you are in the “scissors” rather than the “camera” mode. Choose **File → Open Project**, and open up the movie that you would like to work with. Open one of the movie “clips” from the right side of the screen by clicking on it.

3. Move through the movie clip by moving the triangle that is below the movie screen. Each time that you have a good (clearly focused) stoma visible in the frame, Choose **File → Save Frame**. Choose the pict format, give a suitable name to the frame, and click the Save button. Repeat this process until you have saved enough frames to allow you to measure the number of stomata that you need. Close the iMovie application.

4. Now open the ImageJ application and use **File → Open** to open up the frame for a stoma that you would like to measure. Click on the Straight Line Tool that can be found in the box on the upper part of the screen. As you draw a line across the width of the stomatal opening you will see that the computer measures how long the line is (in pixels). Make the line that you want and then record the measurement. You can continue recording your data from as many frames as you wish until you have measured all the stomata you need.

5. You may find that it is convenient to transfer your data into an Excel spreadsheet file for long term storage and to facilitate calculation of the width/length ratios.

For each of the three leaves, record the aperture of 15 individual stomata and then calculate the mean (+/- SE) value for stomatal aperture for each of the three leaves.

**PART B –
ASSESSMENT OF FACTORS THAT INFLUENCE STOMATAL APERTURE**

Your mission for the next two lab periods (25 October and 1 November) will be to carry out a series of experiments to test the effects of some factor(s) that you think may have an influence on stomatal aperture. Today (18 October) you will develop a written (typed) research proposal outlining the experiments that you intend to do, any specific hypotheses that you will be testing, what controls you will be including, and any unusual equipment or supplies that you will need. Turn in this proposal by the end of lab on 18 October.

Do your experiments and *record your observations and results*. *In addition to the raw data that you collect, also include in your notebook some appropriate graphical representation of the data.*

MES Stomata Buffer

50 mM KCl

1 mM CaCl₂

5 mM MES-KOH pH 6.5